AFRL-RX-TY-TR-2009-4621

CHALLENGE OF N95 AND P100 FILTERING FACEPIECE RESPIRATORS WITH PARTICLE CONTAINING VIABLE H1N1

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NIOSH IAA # 09-42
CDC Agreement IAA #09FED905877

December 2009

AIR FORCE RESEARCH LABORATORY
MATERIALS AND MANUFACTURING DIRECTORATE

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REPORT DOCUMENTATION PAGE

Form Approved
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1. REPORT DATE *(DD-MM-YYYY)*	2. REPORT TYPE	3. DATES COVERED *(From - To)*
2-DEC-2009	Final Technical Report	15 Mar-21 Nov 2009

4. TITLE AND SUBTITLE

Challenge of N95 and P100 Filtering Facepiece Respirators with Particles Containing Viable H1N1

5a. CONTRACT NUMBER

NIOSH IAA # 09-42 ; CDC Agreement IAA #09FE

5b. GRANT NUMBER

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)

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5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

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8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)

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10. SPONSOR/MONITOR'S ACRONYM(S)

AFRL/RXQL

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

AFRL-RX-TY-TR-2009-4621

12. DISTRIBUTION/AVAILABILITY STATEMENT

Distribution A: Approved for public release; distribution unlimited.

13. SUPPLEMENTARY NOTES

Ref Public Affairs Case # AFCEC-201415, 1 May 2014. Document contains color images.

14. ABSTRACT

3M1860s (N95) and 3M 8293 (P100) NIOSH-approved filtering facepiece respirators (FFRs) were challenged with aerosolized particles of H1N1 influenza to measure the amount of viable influenza virus that penetrates each device. The test was conducted at the NIOSH-recommended flowrate of 85 LPM using guidance provided by a Department of Defense test standard developed for challenging air purification devices with viable microbial aerosols. The count mode diameter (CMD) particle size of the challenge aerosol was ~0.8 µm, which was created by aerosolizing H1N1 influenza virus in an artificial saliva buffer using the Laboratory-Scale Aerosol Tunnel (LSAT). In addition to the H1N1 challenge, each FFR was also challenged with 0.8-µm inert beads. In these tests the N95 FFR (n = 3) removed > 99% of the viable H1N1 from the air stream and the P100 (n =3) removed > 99.99% of viable H1N1 from the airstream. The percent reductions in mechanical and viable particle counts measured for each FFR using the 0.8-µm bead challenge were equivalent to the percent reduction values measured for like-sized particles containing H1N1, verifying that bioaerosols act as typical particles. These data demonstrate that the N95 and P100 FFR will reduce viable H1N1 aerosol from the airstream at greater than or equal to their rated value.

15. SUBJECT TERMS

aerosol, FFR, H1N1, infection, influenza, N95, P100, respirator, respiratory

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			Joseph D. Wander
U	U	U	SAR	35	19b. TELEPHONE NUMBER *(Include area code)* 850 283 6240

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NIOSH IAA # 09-42
CDC Agreement IAA #09FED905877

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December 2, 2009

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OVERVIEW

H1N1 is a strain of Influenza A virus belonging to the *Orthomyxoviridae* family[1]. The virus is a single-stranded RNA virus containing a nucleocapsid and envelope that is 80–120 nm in diameter[1]. In March and April 2009 a new flu virus of swine origin was first detected in Mexico and the United States. According to the CDC, since the outbreak began in the United States, an increasing number of U.S. states have reported cases of novel H1N1 influenza with associated hospitalizations and deaths. By 3 June 2009 all 50 states in the United States, the District of Columbia and Puerto Rico were reporting cases of novel H1N1 infection. Current CDC interim recommendations to reduce person-to-person H1N1 virus transmission include the use of respiratory protection devices in some situations. Furthermore, in the past decade, respirators have become commonplace among healthcare workers who aim to protect themselves against any number of respiratory pathogens. Research by NIOSH, AFRL and others has demonstrated that filters such as those used in NIOSH-approved filtering facepiece respirators are capable of capturing bioaerosols as predicted by filtration theory and through comparison with inert (non-biological) aerosols. However, continued stakeholder requests for filtration testing with droplet nuclei containing virus particles similar to the novel H1N1 influenza strain seen in the 2009 outbreak revealed an urgent need to conduct additional research to further validate the filtration performance of NIOSH-approved filtering facepiece respirators. The results of this study will be used by NIOSH, CDC, and other national and international public health agencies to support existing recommendations or provide updated guidance on the use of respiratory protection devices to reduce person-to-person transmission of the novel H1N1 virus.

EXECUTIVE SUMMARY

The 3M1860s (N95) and 3M 8293 (P100) NIOSH-approved filtering facepiece respirators (FFRs) were challenged with aerosolized particles of H1N1 influenza to determine the amount of viable influenza virus that penetrates each device. The test was conducted at the NIOSH-recommended flowrate of 85 LPM using guidance provided by a Department of Defense test standard developed for challenging air purification devices with viable microbial aerosols. The count mode diameter (CMD) particle size of the challenge aerosol was ~0.8 µm, which was created by aerosolizing H1N1 influenza virus in an artificial saliva buffer using the Laboratory-Scale Aerosol Tunnel (LSAT). In addition to the H1N1 challenge, each FFR was also challenged with 0.8-µm inert beads. The N95 FFR (n = 3) removed > 99% of the viable H1N1 from the air stream and the P100 (n =3) removed > 99.99% of viable H1N1 from the airstream. The percent reduction in particle counts measured for each FFR using the 0.8-µm bead challenge were equivalent to the H1N1 percent reduction values. These data demonstrate that the N95 and P100 FFR will reduce viable H1N1 aerosol from the airstream at greater than or equal to their rated value.

[1] Universal Virus Database of the International Committee on Taxonomy of Viruses (ICTVdB). http://www.ncbi.nlm.nih.gov/ICTVdb/Ictv/fr-index.htm

1.0 MATERIALS AND METHODS

1.1 Preparation of H1N1 Virus

Influenza A/PR/8/34 VR-1469 (ATCC VR-95) was propagated in embryonic chicken eggs using standard protocols (1). Virus titers were determined using a tissue culture infectious dose assay ($TCID_{50}$) in Madin–Darby canine kidney cells (MDCK; ATCC CCL-34) with cell culture techniques approved by the World Health Organization (1).

1.2 Laboratory-Scale Aerosol Tunnel (LSAT)

The LSAT was designed to challenge air purification systems with viable microbial aerosols and is ideally suited for this study (Figure 1). A complete description, operation instructions, validation report, and accompanying test protocols have been previously described (2). Briefly, the LSAT is composed of 10-cm diameter stainless steel sanitary fittings and a 15-cm diameter filter holder is used to accommodate the FFR. The biological aerosol is generated using a six-jet Collison nebulizer (BGI Inc., Waltham, Mass.). Dilution air, which is conditioned by passing the air through a humidifier, is added through two porous tube diluters (Mott corporation, Farmington, Ct.), one located upstream and the other downstream of the charge neutralizer. The Kr-85 charge neutralizer (TSI Incorporated, Shoreview, Minn.) is required to noralize charges created on particles during aerosolization. Overflow valves are located upstream of the expansion chamber to divert aerosol away from the test specimen. The expansion duct contains three mixing screens which create turbulent flow and allow the aerosol to mix prior to being exposed to the test specimen. Isokinetic sampling ports located upstream and downstream of the sample allow for viable sampling of microbial agents from the airstream and can also be used with traditional particle counters.

A critical mechanical operational element of the LSAT is to ensure the upstream and downstream sampling ports collect the same volume of particles. To validate the performance of the sampling ports, 30 mL of artificial saliva buffer (3) (0.42 g $NaHCO_3$, 0.04 g $MgCl_2$•7 H_2O, 0.13 g $CaCl_2$•H_2O, 7.70 mL 0.2 *M* KH_2PO_4, 12.3 mL 0.2 *M* K_2HPO_4, 0.11g NH_4Cl, 0.19 g KSCN, 0.12 g $(NH_2)_2CO$, 0.88 g NaCl, 1.04 g KCl, 3.00 g mucin (Sigma–Aldrich, St. Louis, Mo., M1778), 1 L deionized water, *p*H 7) was placed in a six-jet Collison nebulizer (BGI Inc., Waltham, Mass.) and attached to the LSAT. Compressed air (30 psi) was added to the Collison nebulizer to start the aerosol flow. Dilution air was added to both porous tube diluters so that the total flow was 85 LPM.

The LSAT was run for 10 minutes then samples were taken alternately from the upstream and downstream ports using an Aerodynamic Particle Sizer (APS, TSI Incorporated, Shoreview, Minn.) Three upstream and three downstream measurements were collected. The port correlation was repeated three additional times using an aerosol of 0.8-µm polystyrene latex beads (PSL) (Thermo Scientific, Waltham, Mass.).

1.3 Preparation of Filtering Facepiece Respirators

Three replicate samples of 3M 1860s (N95) and 3M 8293 (P100) FFRs were glue sealed into 6-inch sample holders. The filters were leak checked by challenging each filter with an aerosol composed of 0.8-µm PSL beads as described above.

1.4 H1N1 Filtration Studies

Prior to each test the LSAT was flushed with HEPA-purified air for 30 minutes, after which a minimum of three APS measurements were taken on the upstream and down-stream port. A leak-checked FFR was loaded into the LSAT using sanitary compression seal fittings. The six-jet Collison nebulizer, containing 1 mL of H1N1 influenza virus (8.6 $\log_{10}$TCID$_{50}$ per mL) suspension diluted into 30 mL of mucin buffer, was attached to the LSAT. The LSAT was configured to direct the aerosol to the overflow and not to the FFR. Compressed air (30 psi) was applied to the Collison nebulizer and dilution air was added to both porous tube diluters so the total flow was 85 LPM. The system was operated for 10 minutes to bring the nebulizer to steady state, whereupon the LSAT overflow valves were reconfigured to allow the viral aerosol to be exposed to the FFR sample for an additional 5 minutes. Viable sampling of the aerosol into upstream and downstream ports was initiated by connecting All-Glass Impingers (AGI-30, Ace Glass, Vineland, N.J.) containing 20 mL of serum-free Eagle's Minimum Essential Medium (sf-EMEM, Hyclone Laboratories Inc, Logan, Utah) supplemented with 1 % pen/strep and 1% L-glutamine (Sigma Aldrich, St. Louis, Mo.) to the LSAT. The AGI-30s were directly attached to the isokinetic sampling ports on the LSAT to minimize particle loss (Figure 2). Sampling was started by opening the valve on the isokinetic sampling port, followed by applying vacuum to the AGI-30, which sampled at ~12.5 LPM. After 5 minutes the isokinetic sampling port was closed, the vacuum was turned off and the AGI-30 was placed on ice until viable plating was performed. A total of six samples (three upstream and three downstream, alternately sampled) were collected for each FFR. The test was repeated five times to completely account for all six FFRs.

1.5 Viable Plating of H1N1 Influenza Virus

The sf-EMEM buffer in the AGI-30s was evaluated for viable H1N1 using a TCID$_{50}$ assay in MDCK cells as described above. The upstream samples were serially diluted 1/10 to 10^{-6}; The10^{-2} – 10^{-6} dilutions were plated in quadruplicate into 24-well tissue culture plates containing a confluent lawn of MDCK cells. The downstream samples for the N95 FFR were serially diluted to 10^{-4} and all stages of the dilutions were plated in quadruplicate. The downstream P100 samples were serially diluted to 10^{-2}, and the 10^{-1} and 10^{-2} samples were plated in quadruplicate. In addition the entire volume of the neat sample for the P100 FFRs was also plated to maximize sensitivity. The plates were incubated for 4 days at 5% CO_2 /37 ºC prior to reading cytopathic effects.

1.6 Data Analysis

Sampling Port Correlation Factor (CF)—Port correlation with 0.8-µm bead studies used the APS particle bins ranging in size from 0.723–0.925 µm. The count in each bin was summed to yield the total particle concentration for each sample. The port correlation for the mucin buffer used the particle concentration that represented the entire measurement range of the APS (0.5–20 µm). The port correlation was determined by calculating the ratio of the average downstream counts to the average upstream counts (see appendix III).

Filtration Efficiency—Upstream and downstream measurements for the 0.8-µm bead study were collected using the 0.723–0.925 µm bins on the APS as described above. Viable virus collected in the upstream and downstream AGI-30s (viable virus per mL of extract) were determined using the Spearman–Karber formula (4). Equation 1 was used to determine the total amount of virus recovered from the each sample (20-mL impinger volume). Viable filtration efficiency (VFE) of the FFRs was determined using equation 2. Particle filtration efficiency (PFE) of the sample was determined using equation 3. For further clarification see appendices II and IV. Prism 5 software (GraphPad Inc., La Jolla, Calif.) was used to determine 95% confidence intervals for the filtration efficiency

Equation 1: Virus concentration/sample* = $L_S = 10^{[L + \log (V)]}$

Where : L = Viable H1N1 expressed in units of $\log_{10}TCID_{50}$/mL
V = sample volume

* If no viable viruses are present ($L = -\infty$) then Ls will be 0.

Equation 2: $VFE = (\sum_{1-n}^{i} [1- (DLs/ULs)/CF] \times 100\%)/n$

Where: DLs = downstream $\log_{10}TCID_{50}$
ULs = upstream $\log_{10}TCID_{50}$
CF = correlation factor
n = number of determinations

Equation 3: $PFE = (\sum_{1-n}^{i} [1- (D/U)/CF] \times 100\%)/n$

Where: U = upstream particle concentration
D = downstream particle concentration

Statistical analysis of penetration data—A two-tailed unpaired *t*-test was used to compare the nonviable (0.8-µm bead) and viable (H1N1 influenza) filtration data for the three replicates of the N95 and P100 FFRs. The average PFE and VFE values for each FFR were loaded into Prism 5 software (GraphPad Inc., La Jolla, Calif.) to perform the *t*-test at the 95% confidence intervals.

2.0 RESULTS

The upstream and downstream ports of the LSAT were demonstrated to be > 99% similar for sampling particles derived from both mucin buffer and 0.8-µm beads (Table 1). The particle size distributions of the mucin buffer sampled from the upstream and downstream ports were also identical (Figure 3).

The 0.8-µm bead challenge of each FFR indicated the glue seal was adequate to prevent leakage of particles around the FFR (Tables 2 and 3). The N95 FFRs removed 99.86% of the beads and the P100 FFR removed 99.999% of the beads from the air stream. Viable challenge results correlated well with the bead penetration data: The N95 FFRs reduced the airborne challenge of viable influenza by > 99% the P100 FFRs achieved > 99.99% removal (Tables 4 and 5). The bead and H1N1 data for both the N95 and P100 FFRs were found not to be statistically different ($p = 0.06$ and $p = 0.52$, respectively).

3.0 DISCUSSION

The data clearly show that both the N95 and the P100 FFRs are effective at removing viable H1N1 particles from the airstream. The filtration efficiency for both FFRs exceeded their rating, as expected for the particle size used for this study—the FFR rating is based on penetration by the most-penetrating particle size (~0.3 µm). The particles used for this study had a CMD centered near 0.8 µm, so their filtration efficiency was higher. As expected the P100 FFR provided better capture of both viable H1N1 and inert particles than the N95 FFR. The N95 FFR did allow significant penetration by H1N1 influenza but this does not suggest the device is inadequate for protecting users from airborne transmission of influenza. To perform the aerosol test the challenge concentration of influenza is intentionally increased to levels higher than would be expected in a normal infectious disease setting—the average of 3.76 $\log_{10}TCID_{50}$ per liter of air used in this study far exceeds values recorded for airborne influenza concentrations in hospital settings (5).

Because the environment in which the test is performed will influence the removal efficiency of the FFR, conditions were carefully selected based on the guidance provided in the test method (2). The critical conditions are flowrate and particle size. The NIOSH standard test rate of 85 LPM was used as the flowrate for all tests performed. The 0.8-µm particle size was selected to simulate the size of particles generated by a human cough. This was a compromise among the varying particle sizes reported to be exhaled by humans (6–13). We chose to focus on particles produced during coughing as this is a clinical symptom of influenza. Yang *et al*., (14) studied the particle distribution produced by coughs from healthy human volunteers and determined that 82% of droplet nuclei generated by coughs fell inside the particle size window of 0.74–2.12 µm. The particle size distribution used for this test had CMD centered on ~0.8 µm, which was produced by delivering the virus in artificial saliva. While it can be argued that other particle sizes and/or solutions could be used, we consider these particles representative of human respiratory secretions. The same particle size is also used in an ASTM method developed to load surfaces with H1N1 particles that are representative of human respiratory secretions (15).

A comparison of the viable H1N1 penetration and the nonviable bead penetration demonstrates that both provide equivalent filtration efficiency. Thus it can be concluded that the presence of infectious microorganisms does not influence filtration efficiency of the FFR. This same phenomenon has been demonstrated by other researchers using different microbial agents (14, 15). This is an important consideration because experiments aerosolizing highly infections microbial agents such as influenza are expensive and difficult to perform. Undoubtedly it is comforting for healthcare workers and others who use FFRs to see data demonstrating that FFRs filter out viable threat agents. However, filtration theory is very well understood (18) and the applicability of viable filtration data seems to fill an occupational, rather than a scientific niche. Better education of FFR users is needed to help them understand that filtration is solely based on physics and not whether the particle carries a pathogen.

Another caveat of these data that must be considered is that only the performance of the filtration media was evaluated. To achieve expected levels of respiratory protection by a device, a good fit must also be achieved. It is imperative that an OSHA-regulated FFR fit test program be implemented by any organization with a respiratory protection program.

4.0 SUMMARY

N95 and P100 FFRs were shown to be effective at removing viable H1N1 from an airstream. The P100 provided filtration efficiency two orders of magnitude higher than the N95 FFR. The performance of both devices for filtering H1N1 influenza particles was expected based on filtration theory. The study evaluated only the filtration performance of the media, and a proper fit is required to achieve adequate performance of the device. However, with a proper fit, both devices should reduce inhalation exposures to airborne H1N1 aerosols.

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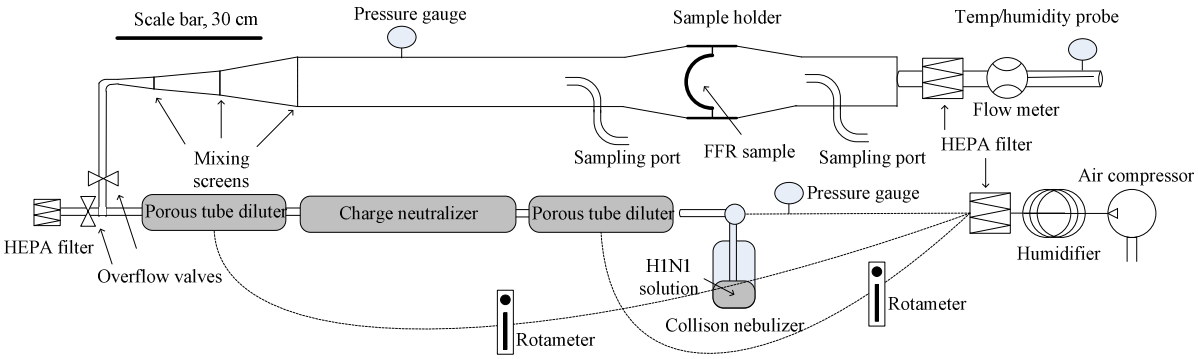

Figure 1. The Laboratory-Scale Aerosol Tunnel (LSAT)

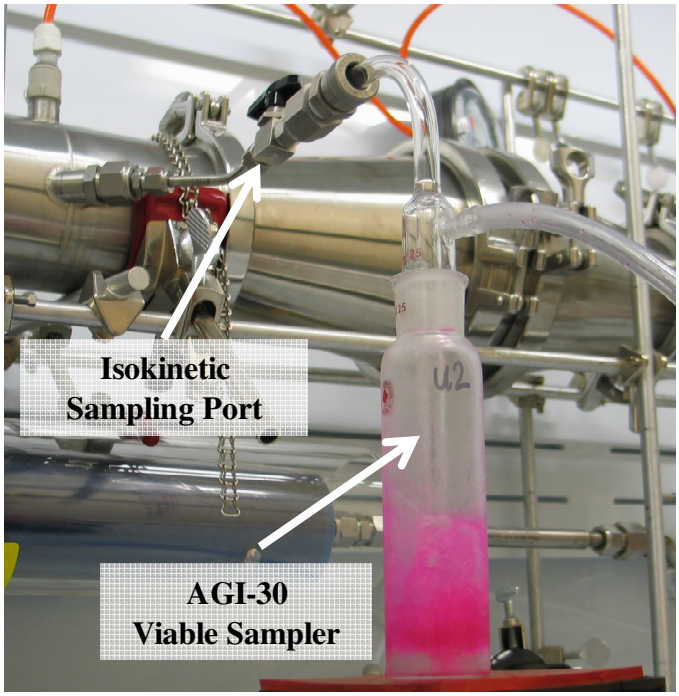

Figure 2. Viable sampling from the LSAT into an AGI-30

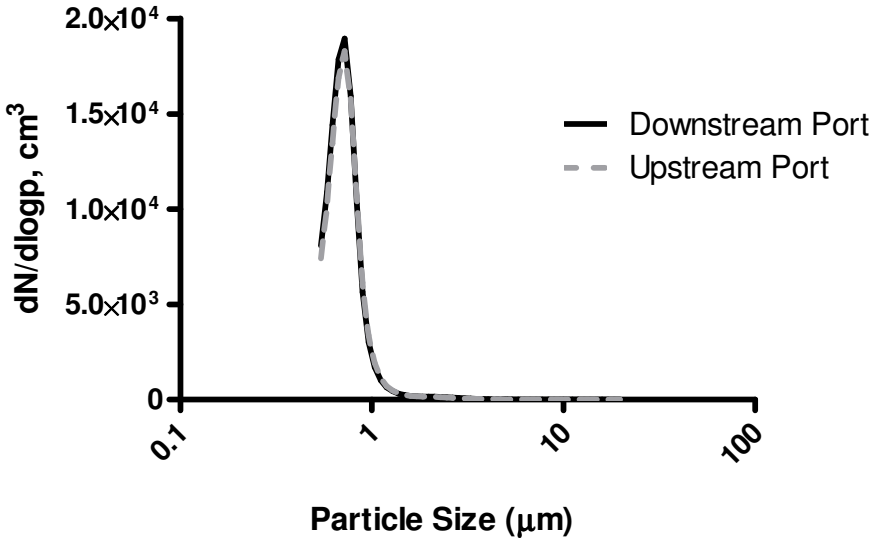

Figure 3. Size Distribution of Artificial Saliva Buffer Aerosolized in LSAT Using the Aerodynamic Particle Sizer Data

Table 1. LSAT Upstream–Downstream Sampling Port Correlation

Aerosol	Particle Concentration (dN/dlogp, cm^3)		Correlation Factor
	Upstream Port	Downstream Port	
Artificial Saliva Buffer	$7.38 \times 10^3 \pm 3.46 \times 10^2$	$7.31 \times 10^3 \pm 1.06 \times 10^2$	1.01
0.8-µm Beads	$6.20 \times 10^3 \pm 1.57 \times 10^2$	$6.25 \times 10^3 \pm 1.31 \times 10^2$	0.99
0.8-µm Beads	$1.00 \times 10^4 \pm 3.26 \times 10^2$	$1.00 \times 10^4 \pm 3.60 \times 10^2$	1.00
0.8-µm Beads	$6.46 \times 10^3 \pm 8.58 \times 10^1$	$6.45 \times 10^3 \pm 1.36 \times 10^2$	1.00
Average			1.00

Table 2. Challenge of Filtering Facepiece Respirators with 0.8-µm Beads (d*N*/dlog*p*, cm^3 for size range 0.723–0 .965 µm)

3M 1860s (N95)	Test 1		Test 2		Test 3	
	Upstream	Downstream	Upstream	Downstream	Upstream	Downstream
Sample #1	7.16×10^3	11.17	7.67×10^3	6.56	8.09×10^3	5.63
Sample #2	8.31×10^3	19.01	8.44×10^3	22.59	8.56×10^3	24.99
Sample #3	1.11×10^4	9.57	1.12×10^4	10.30	1.16×10^4	9.47

3M 8293 (P100)	Test 1		Test 2		Test 3	
	Upstream	Downstream	Upstream	Downstream	Upstream	Downstream
Sample #1	1.70×10^4	ND*	1.76×10^4	ND	1.83×10^4	ND
Sample #2	1.45×10^4	0.10	1.48×10^4	0.32	1.63×10^4	0.48
Sample #3	4.71×10^4	ND	5.55×10^4	ND	No data	ND

*No particles detected

Table 3. Challenge of Filtering Facepiece Respirators with 0.8-µm Beads—Percent Reduction

3M 1860s (N95)	Test 1	Test 2	Test 3	Average	L 95% CI	U 95% CI
Sample #1	99.84%	99.91%	99.93%	99.89%	99.78%	99.99%
Sample #2	99.77%	99.73%	99.71%	99.74%	99.66%	99.81%
Sample #3	99.91%	99.91%	99.92%	99.91%	99.90%	99.93%

3M 8293 (P100)	Test 1	Test 2	Test 3	Average	L 95% CI	U 95% CI
Sample #1	> 99.999%	> 99.999%	> 99.999%	> 99.999%	-----*	-----
Sample #2	99.999%	99.998%	99.997%	99.998%	99.996%	99.999%
Sample #3	> 99.999%	> 99.999%	> 99.999%	> 99.999%	-----	-----

* Statistical analysis cannot be completed when replicate data have identical values

Table 4. Challenge of Filtering Facepiece Respirators with H1N1 influenza ($Log_{10}TCID_{50}$ per sample)

3M 1860s (N95)	Test 1		Test 2		Test 3	
	Upstream	Downstream	Upstream	Downstream	Upstream	Downstream
Sample #1	5.6	3.6	5.6	3.3	5.6	3.6
Sample #2	5.3	3.3	5.05	3.3	5.3	2.8
Sample #3	5.55	2.8	6.05	3.3	5.8	3.55

3M 8293 (P100)	Test 1		Test 2		Test 3	
	Upstream	Downstream	Upstream	Downstream	Upstream	Downstream
Sample #1	5.8	ND	5.3	ND	5.3	0.88
Sample #2	5.55	0.97	5.3	ND	5.55	ND
Sample #3	5.55	ND	5.55	ND	5.55	ND

*No viable virus detected

Table 5. Challenge of Filtering Facepiece Respirators with H1N1 influenza—Percent Reduction

3M 1860s (N95)	Sample 1	Sample 2	Sample 3	Average	L 95% CI	U 95% CI
Sample #1	99.00%	99.44%	99.00%	99.15%	98.52%	99.77%
Sample #2	99.00%	98.22%	99.68%	98.97%	97.15%	99.999%
Sample #3	99.82%	99.82%	99.44%	99.69%	99.17%	99.999%

3M 8293 (P100)	Sample 1	Sample 2	Sample 3	Average	L 95% CI	U 95% CI
Sample #1	> 99.999%	> 99.999%	99.996%	†99.998%	99.994%	99.999%
Sample #2	99.997%	> 99.999%	> 99.999%	†99.998%	99.995%	99.999%
Sample #3	> 99.999%	> 99.999%	> 99.999%	> 99.999%	-----*	-----

*Statistical analysis cannot be completed when replicate data have identical values
† Detection limit of 99.999% was used to calculate averages for samples that had no detectable virus

Appendix I – H1N1 influenza aerosol run forms

Test Samples:							
Sample:	N95 FFR: 3M1860s FFR - #1		Lot # :	17086			
	Dimension:	Full device, glue sealed into 6" circular LSAT mount					
Test stand:		Laboratory Scale Aerosol Tunnel					
Aerosolizer:		6-jet Collison nebulizer; only one used					
Biological collector:		AGI30 impingers (1 per port)					
Microorganism:							
Genus species & ATCC number :		H1N1 Influenza A/PR/8/34 VR-1469 (ATCC VR-95H1N1)					
Preparation method		Embryonic eggs according to WHO protocol					
Reagents:							
Nebulization fluid:		mucin buffer					
Collection buffer:		serum free EMEM					
Dilution buffer:		serum free EMEM					
Media:		EMEM supplemented with serum, pen/strep, and glutamine					
Experimental:							
Pressure drop:	Start:	.35 in water	Middle:	.4 in water		End:	.45 in wate
Temperature	Start:	23.5 C	Middle:	23 C		End:	23 C
Humidity	Start:	32.9% RH	Middle:	34% RH		End:	33.9% RH
System flow rate:	85 SLPM						
Prequilibration time prior to sampling:		15 minutes (10 minutes to overflow and 5 minutes to sample)					
Number of Replicates:	Background	1 per port	100% Correlation:	1 per port		Challenge:	3 per port
Flow rates for biological collectors:		AGI30s sampled at ~12.5 LPM					
Background	5 SLPM Used APS		100% Correlation:	5 SLPM Used APS		Challenge	~12.5 SLPM
Challenge test sampling times for upstream and down stream collectors							
Background:	Upstream:	3 min/APS	Downstream:	3 min/APS			
Correlation:	Upstream:	3 min/APS	Downstream:	3 min/APS			
Challenge #1:	Upstream:	5 min/AGI-30	Downstream:	5 min/AGI-30			
Challenge #2:	Upstream:	5 min/AGI-30	Downstream:	5 min/AGI-30			
Challenge #3:	Upstream:	5 min/AGI-30	Downstream:	5 min/AGI-30			

Test Samples:								
Sample:	N95 FFR: 3M1860s FFR - #2			Lot # :	17086			
	Dimension:	Full device, glue sealed into 6" circular LSAT mount						
Test stand:		Laboratory Scale Aerosol Tunnel						
Aerosolizer:		6-jet Collison nebulizer; only one used						
Biological collector:		AGI30 impingers (1 per port)						
Microorganism:								
Genus species & ATCC number :		H1N1 Influenza A/PR/8/34 VR-1469 (ATCC VR-95H1N1)						
Preparation method		Embryonic eggs according to WHO protocol						
Reagents:								
Nebulization fluid:		mucin buffer						
Collection buffer:		serum free EMEM						
Dilution buffer:		serum free EMEM						
Media:		EMEM supplemented with serum, pen/strep, and glutamine						
Experimental:								
Pressure drop:	Start:	.4 in water		Middle:	.4 in water		End:	.45 in wate
Temperature	Start:	24.1 C		Middle:	24 C		End:	23.9 C
Humidity	Start:	31.6% RH		Middle:	31.9% RH		End:	32% RH
System flow rate:	85 SLPM							
Prequilibration time prior to sampling:		15 minutes (10 minutes to overflow and 5 minutes to sample)						
Number of Replicates:	Background	1 per port		100% Correlation:	1 per port		Challenge:	3 per port
Flow rates for biological collectors:		AGI30s sampled at ~12.5 LPM						
Background	5 SLPM Used APS			100% Correlation:	5 SLPM Used APS		Challenge	~12.5 SLPM
Challenge test sampling times for upstream and down stream collectors								
Background:	Upstream:	3 min/APS		Downstream:	3 min/APS			
Correlation:	Upstream:	3 min/APS		Downstream:	3 min/APS			
Challenge #1:	Upstream:	5 min/AGI-30		Downstream:	5 min/AGI-30			
Challenge #2:	Upstream:	5 min/AGI-30		Downstream:	5 min/AGI-30			
Challenge #3:	Upstream:	5 min/AGI-30		Downstream:	5 min/AGI-30			

Test Samples:								
Sample:	N95 FFR: 3M1860s FFR - #3			Lot # :	17086			
	Dimension:	Full device, glue sealed into 6" circular LSAT mount						
Test stand:		Laboratory Scale Aerosol Tunnel						
Aerosolizer:		6-jet Collison nebulizer; only one used						
Biological collector:		AGI30 impingers (1 per port)						
Microorganism:								
Genus species & ATCC number :		H1N1 Influenza A/PR/8/34 VR-1469 (ATCC VR-95H1N1)						
Preparation method		Embryonic eggs according to WHO protocol						
Reagents:								
Nebulization fluid:		mucin buffer						
Collection buffer:		serum free EMEM						
Dilution buffer:		serum free EMEM						
Media:		EMEM supplemented with serum, pen/strep, and glutamine						
Experimental:								
Pressure drop:	Start:	.35 in water		Middle:	.4 in water		End:	.45 in wate
Temperature	Start:	24.2 C		Middle:	23.9 C		End:	23.9 C
Humidity	Start:	31.6% RH		Middle:	31.9% RH		End:	32% RH
System flow rate:	85 SLPM							
Prequilibration time prior to sampling:		15 minutes (10 minutes to overflow and 5 minutes to sample)						
Number of Replicates:	Background	1 per port		100% Correlation:	1 per port		Challenge:	3 per port
Flow rates for biological collectors:		AGI30s sampled at ~12.5 LPM						
Background	5 SLPM Used APS		100% Correlation:	5 SLPM Used APS		Challenge	~12.5 SLPM	
Challenge test sampling times for upstream and down stream collectors								
Background:	Upstream:	3 min/APS		Downstream:	3 min/APS			
Correlation:	Upstream:	3 min/APS		Downstream:	3 min/APS			
Challenge #1:	Upstream:	5 min/AGI-30		Downstream:	5 min/AGI-30			
Challenge #2:	Upstream:	5 min/AGI-30		Downstream:	5 min/AGI-30			
Challenge #3:	Upstream:	5 min/AGI-30		Downstream:	5 min/AGI-30			

Test Samples:								
Sample:	P100 FFR: 3M 8293 FFR - #1			Lot # :	19135			
	Dimension:	Full device, glue sealed into 6" circular LSAT mount						
Test stand:		Laboratory Scale Aerosol Tunnel						
Aerosolizer:		6-jet Collison nebulizer; only one used						
Biological collector:		AGI30 impingers (1 per port)						
Microorganism:								
Genus species & ATCC number :		H1N1 Influenza A/PR/8/34 VR-1469 (ATCC VR-95H1N1)						
Preparation method		Embryonic eggs according to WHO protocol						
Reagents:								
Nebulization fluid:		mucin buffer						
Collection buffer:		serum free EMEM						
Dilution buffer:		serum free EMEM						
Media:		EMEM supplemented with serum, pen/strep, and glutamine						
Experimental:								
Pressure drop:	Start:	.55 in water		Middle:	.55 in water		End:	.6 in water
Temperature	Start:	22.7 C		Middle:	22.5 C		End:	22.7 C
Humidity	Start:	26.3% RH		Middle:	23.8% RH		End:	23.6% RH
System flow rate:	85 SLPM							
Prequilibration time prior to sampling:		15 minutes (10 minutes to overflow and 5 minutes to sample)						
Number of Replicates:	Background	1 per port		100% Correlation:	1 per port		Challenge:	3 per port
Flow rates for biological collectors:		AGI30s sampled at ~12.5 LPM						
Background	5 SLPM Used APS		100% Correlation:	5 SLPM Used APS		Challenge	~12.5 SLPM	
Challenge test sampling times for upstream and down stream collectors								
Background:	Upstream:	3 min/APS		Downstream:	3 min/APS			
Correlation:	Upstream:	3 min/APS		Downstream:	3 min/APS			
Challenge #1:	Upstream:	5 min/AGI-30		Downstream:	5 min/AGI-30			
Challenge #2:	Upstream:	5 min/AGI-30		Downstream:	5 min/AGI-30			
Challenge #3:	Upstream:	5 min/AGI-30		Downstream:	5 min/AGI-30			

Test Samples:								
Sample:	P100 FFR: 3M 8293 FFR - #2			Lot # :	19135			
	Dimension:	Full device, glue sealed into 6" circular LSAT mount						
Test stand:		Laboratory Scale Aerosol Tunnel						
Aerosolizer:		6-jet Collison nebulizer; only one used						
Biological collector:		AGI30 impingers (1 per port)						
Microorganism:								
Genus species & ATCC number :		H1N1 Influenza A/PR/8/34 VR-1469 (ATCC VR-95H1N1)						
Preparation method		Embryonic eggs according to WHO protocol						
Reagents:								
Nebulization fluid:		mucin buffer						
Collection buffer:		serum free EMEM						
Dilution buffer:		serum free EMEM						
Media:		EMEM supplemented with serum, pen/strep, and glutamine						
Experimental:								
Pressure drop:	Start:	.6 in water		Middle:	.65 in water		End:	.65 in wate
Temperature	Start:	23.3 C		Middle:	23.4 C		End:	23.4 C
Humidity	Start:	25.8% RH		Middle:	23.4% RH		End:	25.3% RH
System flow rate:	85 SLPM							
Prequilibration time prior to sampling:		15 minutes (10 minutes to overflow and 5 minutes to sample)						
Number of Replicates:	Background	1 per port		100% Correlation:	1 per port		Challenge:	3 per port
Flow rates for biological collectors:		AGI30s sampled at ~12.5 LPM						
Background	5 SLPM Used APS		100% Correlation:	5 SLPM Used APS		Challenge	~12.5 SLPM	
Challenge test sampling times for upstream and down stream collectors								
Background:	Upstream:	3 min/APS		Downstream:	3 min/APS			
Correlation:	Upstream:	3 min/APS		Downstream:	3 min/APS			
Challenge #1:	Upstream:	5 min/AGI-30		Downstream:	5 min/AGI-30			
Challenge #2:	Upstream:	5 min/AGI-30		Downstream:	5 min/AGI-30			
Challenge #3:	Upstream:	5 min/AGI-30		Downstream:	5 min/AGI-30			

Test Samples:								
Sample:	P100 FFR: 3M 8293 FFR - #3			Lot # :	19135			
	Dimension:	Full device, glue sealed into 6" circular LSAT mount						
Test stand:		Laboratory Scale Aerosol Tunnel						
Aerosolizer:		6-jet Collison nebulizer; only one used						
Biological collector:		AGI30 impingers (1 per port) containing 20 mL of collection buffer						
Microorganism:								
Genus species & ATCC number :		H1N1 Influenza A/PR/8/34 VR-1469 (ATCC VR-95H1N1)						
Preparation method		Embryonic eggs according to WHO protocol						
Reagents:								
Nebulization fluid:		mucin buffer						
Collection buffer:		serum free EMEM						
Dilution buffer:		serum free EMEM						
Media:		EMEM supplemented with serum, pen/strep, and glutamine						
Experimental:								
Pressure drop:	Start:	.55 in water		Middle:	.55 in water		End:	.6 in water
Temperature	Start:	24.9 C		Middle:	24.9 C		End:	25 C
Humidity	Start:	26.3% RH		Middle:	26.3% RH		End:	26% RH
System flow rate:	85 SLPM							
Prequilibration time prior to sampling:		15 minutes (10 minutes to overflow and 5 minutes to sample)						
Number of Replicates:	Background	1 per port		100% Correlation:	1 per port		Challenge:	3 per port
Flow rates for biological collectors:		AGI30s sampled at ~12.5 LPM						
Background	5 SLPM Used APS		100% Correlation:		5 SLPM Used APS		Challenge	~12.5 SLPM
Challenge test sampling times for upstream and down stream collectors								
Background:	Upstream:	3 min/APS		Downstream:	3 min/APS			
Correlation:	Upstream:	3 min/APS		Downstream:	3 min/APS			
Challenge #1:	Upstream:	5 min/AGI-30		Downstream:	5 min/AGI-30			
Challenge #2:	Upstream:	5 min/AGI-30		Downstream:	5 min/AGI-30			
Challenge #3:	Upstream:	5 min/AGI-30		Downstream:	5 min/AGI-30			

Appendix II – H1N1 raw data

Sample: **3M 1860S #1**

Plating Results:

Dilution	Upstream 1				Upstream 2				Upstream 3			
-2	X	X	X	X	X	X	X	X	X	X	X	X
-3	X	X	X	X	X	X	X	X	X	X	X	X
-4	X	X	X	o	X	o	X	o	o	X	X	o
-5	o	o	o	o	X	o	o	o	o	X	o	o
-6	o	o	o	o	o	o	o	o	o	o	o	o

Dilution	Downstream 1				Downstream 2				Downstream 3			
Undiluted	X	X	X	X	X	X	X	X	X	X	X	X
-1	X	X	X	X	X	X	X	X	X	X	X	X
-2	X	X	o	o	X	X	o	o	X	X	o	X
-3	o	X	o	o	o	o	o	o	o	o	o	o
-4	o	o	o	o	o	o	o	o	o	o	o	o

x = cytopathic effect, o = healthy cells

Data:

LOG $TCID_{50}$/mL (*L*)	**4.25**	**4.25**	**4.25**	**2.25**	**2.00**	**2.25**
Sample volume (*V*)	20	20	20	20	20	20
Log *V*	1.30	1.30	1.30	1.30	1.30	1.30
Log titer per sample	5.6	5.6	5.6	3.6	3.3	3.6
	ULs1	*ULs2*	*ULs3*	*DLs1*	*DLs2*	*DLs3*
$TCID_{50}$ infectious dose units (*Ls*)	3.56E+05	3.56E+05	3.56E+05	3.56E+03	2.00E+03	3.56E+03
Average	3.56E+05			3.04E+03		
stdev	0			0.14		

Percent Reduction (VRE)

1-(*DLs1* ÷ *ULs1* ÷ *CF**) X 100%	99.000%
1-(*DLs2* ÷ *ULs2* ÷ *CF**) X 100%	99.438%
1-(*DLs3* ÷ *ULs3* ÷ *CF**) X 100%	99.000%
VRE	**99.146%**

*CF (Correlation Factor) = 1

Aerosol concentration (Log titer per Liter of air): 3.76

Sample: **3M 1860S #2**

Plating Results:

Dilution	Upstream 1				Upstream 2				Upstream 3			
-2	X	X	X	X	X	X	X	X	X	X	X	X
-3	X	X	X	X	X	X	X	X	X	X	X	X
-4	o	X	X	o	o	o	o	X	o	X	X	o
-5	o	o	o	o	o	X	o	o	o	o	o	o
-6	o	o	o	o	o	o	o	o	o	o	o	o

Dilution	Downstream 1				Downstream 2				Downstream 3			
Undiluted	X	X	X	X	X	X	X	X	X	X	X	X
-1	X	X	X	X	X	X	X	X	X	X	X	X
-2	X	X	o	o	X	X	o	o	o	o	o	o
-3	o	o	o	o	o	o	o	o	o	o	o	o
-4	o	o	o	o	o	o	o	o	o	o	o	o

x = cytopathic effect, o = healthy cells

Data:

	Upstream 1	Upstream 2	Upstream 3	Downstream 1	Downstream 2	Downstream 3
LOG $TCID_{50}$/mL (*L*)	**4.00**	**3.75**	**4.00**	**2.00**	**2.00**	**1.50**
Sample volume (*V*)	20	20	20	20	20	20
Log *V*	1.30	1.30	1.30	1.30	1.30	1.30
Log titer per sample	5.3	5.1	5.3	3.3	3.3	2.8
	ULs1	*ULs2*	*ULs3*	*DLs1*	*DLs2*	*DLs3*
$TCID_{50}$ infectious dose units (*Ls*)	2.00E+05	1.12E+05	2.00E+05	2.00E+03	2.00E+03	6.32E+02
Average	1.71E+05			1.54E+03		
stdev	0.144337567			0.29		

Percent Reduction (VRE)

1-(*DLs1* ÷ *ULs1* ÷ *CF**) X 100%	99.000%
1-(*DLs2* ÷ *ULs2* ÷ *CF**) X 100%	98.222%
1-(*DLs3* ÷ *ULs3* ÷ *CF**) X 100%	99.684%
VRE	**98.968%**

*CF (Correlation Factor) = 1

Aerosol concentration (Log titer per Liter of air): 3.44

Sample: **3M 1860S #3**

Plating Results:

Dilution	Upstream 1				Upstream 2				Upstream 3			
-2	X	X	X	X	X	X	X	X	X	X	X	X
-3	X	X	X	X	X	X	X	X	X	X	X	X
-4	o	X	X	X	X	X	X	X	X	X	X	X
-5	o	o	o	o	o	o	o	X	o	o	o	o
-6	o	o	o	o	o	o	o	o	o	o	o	o

Dilution	Downstream 1				Downstream 2				Downstream 3			
Undiluted	X	X	X	X	X	X	X	X	X	X	X	X
-1	X	X	X	X	X	X	X	X	X	X	X	X
-2	o	o	o	o	o	X	o	X	X	X	o	X
-3	o	o	o	o	o	o	o	o	o	o	o	o
-4	o	o	o	o	o	o	o	o	o	o	o	o

x = cytopathic effect, o = healthy cells

Data:

	Upstream 1	Upstream 2	Upstream 3	Downstream 1	Downstream 2	Downstream 3
LOG $TCID_{50}$/mL (*L*)	**4.25**	**4.75**	**4.50**	**1.50**	**2.00**	**2.25**
Sample volume (*V*)	20	20	20	20	20	20
Log *V*	1.30	1.30	1.30	1.30	1.30	1.30
Log titer per sample	5.6	6.1	5.8	2.8	3.3	3.6
	ULs1	*ULs2*	*ULs3*	*DLs1*	*DLs2*	*DLs3*
$TCID_{50}$ infectious dose units (*Ls*)	3.56E+05	1.12E+06	6.32E+05	6.32E+02	2.00E+03	3.56E+03
Average	7.04E+05			2.06E+03		
stdev	0.25			0.38		

Percent Reduction (VRE)

1-(*DLs1* ÷ *ULs1* ÷ *CF**) X 100%	99.822%
1-(*DLs2* ÷ *ULs2* ÷ *CF**) X 100%	99.822%
1-(*DLs3* ÷ *ULs3* ÷ *CF**) X 100%	99.438%
VRE	**99.694%**

*CF (Correlation Factor) = 1

Aerosol concentration (Log titer per Liter of air): 4.05

Sample: **3M 8293 #1**

Plating Results:

Dilution	Upstream 1				Upstream 2				Upstream 3			
-2	X	X	X	X	X	X	X	X	X	X	X	X
-3	X	X	X	X	X	X	X	X	X	X	X	X
-4	X	X	X	X	o	o	o	X	X	o	X	o
-5	o	o	o	o	o	o	o	X	o	o	o	o
-6	o	o	o	o	o	o	o	o	o	o	o	o

Dilution	Downstream 1				Downstream 2				Downstream 3			
Undiluted	o	o	o	o	o	o	o	o	o	o	o	o
Undiluted	o	o	o	o	o	o	o	o	o	o	o	X
Undiluted	o	o	o	o	o	o	o	o	o	o	o	o
-1	o	o	o	o	o	o	o	o	o	o	o	o
-2	o	o	o	o	o	o	o	o	o	o	o	o

x = cytopathic effect, o = healthy cells

Data:

LOG $TCID_{50}$/mL (*L*)	**4.50**	**4.00**	**4.00**	**0.00**	**0.00**	**-0.42**
Sample volume (*V*)	20	20	20	20	20	20
Log *V*	1.30	1.30	1.30	1.30	1.30	1.30
Log titer per sample	5.8	5.3	5.3	1.3	1.3	0.9
	ULs1	*ULs2*	*ULs3*	*DLs1*	*DLs2*	*DLs3*
$TCID_{50}$ infectious dose units (*Ls*)	6.32E+05	2.00E+05	2.00E+05	0.00E+00	0.00E+00	7.66E+00
Average	3.44E+05			2.55E+00		
stdev	0.288675135			0.24		

Percent Reduction (VRE)

1-(*DLs1* ÷ *ULs1* ÷ *CF**) X 100%	99.999%
1-(*DLs2* ÷ *ULs2* ÷ *CF**) X 100%	99.999%
1-(*DLs3* ÷ *ULs3* ÷ *CF**) X 100%	99.996%
VRE	**99.998%**

*CF (Correlation Factor) = 1

Aerosol concentration (Log titer per Liter of air): 3.74

Sample: **3M 8293 #2**

Plating Results:

Dilution	Upstream 1				Upstream 2				Upstream 3			
-2	X	X	X	X	X	X	X	X	X	X	X	X
-3	X	X	X	X	X	X	X	X	X	X	X	X
-4	X	o	X	X	o	o	o	X	X	X	o	X
-5	o	o	o	o	o	X	o	X	o	o	o	o
-6	o	o	o	o	X	o	o	o	o	o	o	o

Dilution	Downstream 1				Downstream 2				Downstream 3			
Undiluted	o	X	X	o	o	o	o	o	o	o	o	o
Undiluted	o	o	o	o	o	o	o	o	o	o	o	o
Undiluted	o	o	o	o	o	o	o	o	o	o	o	o
-1	o	o	o	o	o	o	o	o	o	o	o	o
-2	o	o	o	o	o	o	o	o	o	o	o	o

x = cytopathic effect, o = healthy cells

Data:

LOG $TCID_{50}$/mL (*L*)	**4.25**	**4.00**	**4.25**	**-0.33**	**0.00**	**0.00**
Sample volume (*V*)	20	20	20	20	20	20
Log *V*	1.30	1.30	1.30	1.30	1.30	1.30
Log titer per sample	5.6	5.3	5.6	1.0	1.3	1.3
	ULs1	*ULs2*	*ULs3*	*DLs1*	*DLs2*	*DLs3*
$TCID_{50}$ infectious dose units (*Ls*)	3.56E+05	2.00E+05	3.56E+05	9.28E+00	0.00E+00	0.00E+00
Average	3.04E+05			3.09E+00		
stdev	0.144337567			0.19		

Percent Reduction (VRE)

1-(*DLs1* ÷ *ULs1* ÷ *CF**) X 100%	99.997%
1-(*DLs2* ÷ *ULs2* ÷ *CF**) X 100%	99.999%
1-(*DLs3* ÷ *ULs3* ÷ *CF**) X 100%	99.999%
VRE	**99.998%**

*CF (Correlation Factor) = 1

Aerosol concentration (Log titer per Liter of air): 3.69

Sample: **3M 8293 #3**

Plating Results:

Dilution	Upstream 1				Upstream 2				Upstream 3			
-2	X	X	X	X	X	X	X	X	X	X	X	X
-3	X	X	X	X	X	X	X	X	X	X	X	X
-4	o	X	X	X	X	X	o	X	o	o	X	X
-5	o	o	o	o	o	o	o	o	o	o	o	o
-6	o	o	o	o	o	o	o	o	o	o	o	o

Dilution	Downstream 1				Downstream 2				Downstream 3			
Undiluted	o	o	o	o	o	o	o	o	o	o	o	o
Undiluted	o	o	o	o	o	o	o	o	o	o	o	o
Undiluted	o	o	o	o	o	o	o	o	o	o	o	o
-1	o	o	o	o	o	o	o	o	o	o	o	o
-2	o	o	o	o	o	o	o	o	o	o	o	o

x = cytopathic effect, o = healthy cells

Data:

LOG $TCID_{50}$/mL (*L*)	**4.25**	**4.25**	**4.00**	**0.00**	**0.00**	**0.00**
Sample volume (*V*)	20	20	20	20	20	20
Log *V*	1.30	1.30	1.30	1.30	1.30	1.30
Log titer per sample	5.6	5.6	5.3	1.3	1.3	1.3
	ULs1	*ULs2*	*ULs3*	*DLs1*	*DLs2*	*DLs3*
$TCID_{50}$ infectious dose units (*Ls*)	3.56E+05	3.56E+05	2.00E+05	0.00E+00	0.00E+00	0.00E+00
Average	3.04E+05			0.00E+00		
stdev	0.144337567			0.00		

Percent Reduction (VRE)

1-(*DLs1* ÷ *ULs1* ÷ *CF**) X 100%	99.999%
1-(*DLs2* ÷ *ULs2* ÷ *CF**) X 100%	99.999%
1-(*DLs3* ÷ *ULs3* ÷ *CF**) X 100%	99.999%
VRE	**99.999%**

*CF (Correlation Factor) = 1

Aerosol concentration (Log titer per Liter of air): 3.69

Appendix III – Port correlation data

Mucin Buffer Sampling Port Correlation		
Samples	Upstream	Downstream
Particle concentration	6904.34	7156.13
Particle concentration	6999.7	7210.74
Particle concentration	7135.61	7226.24
Particle concentration	7229.31	7318.97
Particle concentration	7312.19	7335.87
Particle concentration	7312.86	7486.55
Particle concentration	7896.61	7430.33
Particle concentration	7892.69	7338.32
Particle concentration	7897.78	7265.31
Particle concentration	7464.38	
Particle concentration	7385.25	
Particle concentration	7153.51	
Average	7382.02	7307.61
Stdev	346.23	105.88
CV	4.7%	1.4%
Correlation Factor	**1.01**	**0.99**

0.8 µm Beads #1 Port Correlation		
Samples	Upstream	Downstream
Concentration (0.723 - 0.965)	6067.07	6114.21
Concentration (0.723 - 0.965)	5972.61	6099.33
Concentration (0.723 - 0.965)	6061.80	6098.57
Concentration (0.723 - 0.965)	6235.45	6196.58
Concentration (0.723 - 0.965)	6181.80	6251.20
Concentration (0.723 - 0.965)	6193.70	6261.28
Concentration (0.723 - 0.965)	6261.28	6421.90
Concentration (0.723 - 0.965)	6308.22	6349.80
Concentration (0.723 - 0.965)	6503.77	6426.02
Average	6198.41	6246.54
Stdev	157.25	131.07
CV	2.5%	2.1%
Correlation Factor	**0.99**	**1.01**

0.8 µm Beads #2 Port Correlation		
Samples	Upstream	Downstream
Concentration (0.723 - 0.965)	9720.10	9437.19
Concentration (0.723 - 0.965)	9673.91	9600.57
Concentration (0.723 - 0.965)	9516.48	9709.92
Concentration (0.723 - 0.965)	9892.32	10168.11
Concentration (0.723 - 0.965)	10028.63	10207.19
Concentration (0.723 - 0.965)	10117.62	10185.11
Concentration (0.723 - 0.965)	10478.37	10423.00
Concentration (0.723 - 0.965)	10307.70	10361.08
Concentration (0.723 - 0.965)	10286.86	10278.80
Average	10002.44	10041.22
Stdev	326.05	360.15
CV	3.3%	3.6%
Correlation Factor	**1.00**	**1.00**

0.8 µm Beads #3 Port Correlation		
Samples	Upstream	Downstream
Concentration (0.723 - 0.965)	6330.79	6158.86
Concentration (0.723 - 0.965)	6366.79	6300.36
Concentration (0.723 - 0.965)	6385.70	6436.58
Concentration (0.723 - 0.965)	6436.58	6529.41
Concentration (0.723 - 0.965)	6544.96	6537.57
Concentration (0.723 - 0.965)	6524.03	6536.32
Concentration (0.723 - 0.965)	6533.73	6540.16
Concentration (0.723 - 0.965)	6512.22	6551.10
Concentration (0.723 - 0.965)	6550.15	6494.94
Average	6464.99	6453.92
Stdev	85.76	136.47
CV	1.3%	2.1%
Correlation Factor	**1.00**	**1.00**

Appendix IV – 0.8µm bead challenge data

3M 1860 #1	Particle Concentration (0.723 - 0.965µm bins)			
Samples	Upstream	Upstream Average (U)	Downstream	Downstream Average (D)
Sample 1	7159.633	7161.01	10.94378	11.167778
Sample 1	7125.168		13.53573	
Sample 1	7198.23		9.023824	
Sample 2	7575.206	7666.70	6.623876	6.559873067
Sample 2	7606.697		7.0078581	
Sample 2	7818.182		6.0478851	
Sample 3	8112.409	8086.46	5.183895	5.631889033
Sample 3	8002.591		5.663893	
Sample 3	8144.382		6.0478791	

Percent Reduction	
1-(*D1 ÷ U1 ÷ CF**) X 100%	99.84%
1-(*D2 ÷ U2 ÷ CF**) X 100%	99.91%
1-(*D3 ÷ U3 ÷ CF**) X 100%	99.93%
Average Percent Reduction	**99.90%**

*CF (Correlation Factor) = 1

3M 1860 #2	Particle Concentration (0.723 - 0.965µm bins)			
Samples	Upstream	Upstream Average (U)	Downstream	Downstream Average (D)
Sample 1	8304.7	8307.16	19.2956401	19.00764607
Sample 1	8317.369		18.047643	
Sample 1	8299.422		19.6796551	
Sample 2	8427.956	8438.10	23.327493	22.59153233
Sample 2	8403.572		21.31161	
Sample 2	8482.779		23.135494	
Sample 3	8515.413	8559.51	24.1915001	24.99148473
Sample 3	8629.18		24.3834901	
Sample 3	8533.939		26.399464	

Percent Reduction	
1-(*D1 ÷ U1 ÷ CF**) X 100%	99.77%
1-(*D2 ÷ U2 ÷ CF**) X 100%	99.73%
1-(*D3 ÷ U3 ÷ CF**) X 100%	99.71%
Average Percent Reduction	**99.74%**

*CF (Correlation Factor) = 1

3M 1860 #3	Particle Concentration (0.723 - 0.965µm bins)			
Samples	Upstream	Upstream Average (U)	Downstream	Downstream Average (D)
Sample 1	11048.51	11079.46	9.21582	9.5678134
Sample 1	11054.08		8.639834	
Sample 1	11135.77		10.8477862	
Sample 2	11077.31	11218.46	10.271792	10.30379137
Sample 2	11208.83		10.6557821	
Sample 2	11369.24		9.9838	
Sample 3	11547.51	11566.36	9.8878081	9.4718074
Sample 3	11552.41		9.599796	
Sample 3	11599.16		8.9278181	

Percent Reduction	
1-(*D1 ÷ U1 ÷ CF**) X 100%	99.91%
1-(*D2 ÷ U2 ÷ CF**) X 100%	99.91%
1-(*D3 ÷ U3 ÷ CF**) X 100%	99.92%
Average Percent Reduction	**99.91%**

*CF (Correlation Factor) = 1

3M 8293 #1	Particle Concentration (0.723 - 0.965µm bins)			
Samples	Upstream	Upstream Average (U)	Downstream	Downstream Average (D)
Sample 1	16954.99	17013.13	0	0
Sample 1	17008.75		0	
Sample 1	17075.66		0	
Sample 2	17438.63	17593.28	0	0
Sample 2	17635.71		0	
Sample 2	17705.51		0	
Sample 3	18233.01	18313.20	0	0
Sample 3	18369.61		0	
Sample 3	18336.98		0	

Percent Reduction	
1-(*D1* ÷ *U1* ÷ *CF**) X 100%	99.999%
1-(*D2* ÷ *U2* ÷ *CF**) X 100%	99.999%
1-(*D3* ÷ *U3* ÷ *CF**) X 100%	99.999%
Average Percent Reduction	99.999%

*CF (Correlation Factor) = 1

3M 8293 #2	Particle Concentration (0.723 - 0.965µm bins)			
Samples	Upstream	Upstream Average (U)	Downstream	Downstream Average (D)
Sample 1	14396.74	14518.53	0	0.0959981
Sample 1	14364.67		0.1919962	
Sample 1	14794.17		0.0959981	
Sample 2	14794.94	14755.99	0.287994	0.319993433
Sample 2	14727.06		0.3839922	
Sample 2	14745.98		0.2879941	
Sample 3	16013.82	16324.18	0.7679842	0.479990233
Sample 3	16349.62		0.3839922	
Sample 3	16609.11		0.2879943	

Percent Reduction	
1-(*D1* ÷ *U1* ÷ *CF**) X 100%	99.999%
1-(*D2* ÷ *U2* ÷ *CF**) X 100%	99.998%
1-(*D3* ÷ *U3* ÷ *CF**) X 100%	99.997%
Average Percent Reduction	**99.998%**

*CF (Correlation Factor) = 1

3M 8293 #3	Particle Concentration (0.723 - 0.965µm bins)			
Samples	Upstream	Upstream Average (U)	Downstream	Downstream Average (D)
Sample 1	45778.777	47075.52	0	0
Sample 1	46754.683		0	
Sample 1	48693.114		0	
Sample 2	54694.245	55452.70	0	0
Sample 2	55500.955		0	
Sample 2	56162.899		0	
Sample 3	No data	No data	0	0
Sample 3	No data		0	
Sample 3	No data		0	

Percent Reduction	
1-(*D1* ÷ *U1* ÷ *CF**) X 100%	99.999%
1-(*D2* ÷ *U2* ÷ *CF**) X 100%	99.999%
1-(*D3* ÷ *U3* ÷ *CF**) X 100%	99.999%
Average Percent Reduction	99.999%

*CF (Correlation Factor) = 1

LIST OF ABBREVIATIONS, ACRONYMS AND SYMBOLS

AFRL	Air Force Research Laboratory
AGI-30	all-glass impinger with a 30-mL reservoir
APS	aerosol particle sizer
ASTM	ASTM International (formerly American Society for Testing Materials)
CDC	Center for Disease Control
CF	correlation factor (for sampling ports [p.4])
cm	centimeter
CMD	count mode diameter
CO_2	carbon dioxide
FFR	filtering facepiece respirator
g	gram(s)
H1N1	a strain of influenza A identified by its hemagglutinin and neuraminindase
Kr-85	a radioactive isotope of krypton
L	liter
LPM	liters per minute
LSAT	Laboratory-Scale Aerosol Tunnel
MDCK	Madin–Darby canine kidney cells
mL	milliliter
n	number of samples tested at the conditions specified
N95	an oil-sensitive respirator that captures ≥95% of challenging 300-nm particles
NIOSH	National Institute for Occupational Safety and Health
nm	nanometer $= 10^{-9}$ meter
P100	an oil-resistant respirator that captures ≥99.97% of challenging 300-nm particles
pen/strep	a mixture of penicillin and streptomycin used to suppress bacterial colonization
PFE	particle filtration efficiency
PSL	polystyrene latex (beads)
RNA	ribonucleic acid
Sf-EMEM	serum-free Eagle's minimum essential medium
$TCID_{50}$	median infective dose in tissue culture
VFE	viable filtration efficiency
µm	micrometer $= 10^{-6}$ meter
°C	temperature in degrees Celsius